THE ELECTROSTATIC ENVIRONMENT, NATIVE STATE TOPOLOGY AND INITIAL CONFORMATION ENSEMBLE DISTINCTLY GUIDE RNA THROUGH PREFERRED FOLDING PATHWAYS

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While it is generally agreed that large RNA molecules typically follow multiple parallel pathways through rugged folding landscapes, the forces that partition molecules among these pathways are poorly understood. Direct influences on the physical forces that govern the folding reaction include native state topology, electrostatic environment and the ensemble of conformations present in the prefolded RNA. The relative contribution of these factors on folding flux partitioning was investigated for the Mg2+-mediated folding of the Tetrahymena ribozyme by coordinated time-resolved hydroxyl radical footprinting and kinetic modeling [A.L. Laederach, I. Shcherbakova, I., M.P. Liang, M. Brenowitz & R.B. Altman (2006) J. Mol. Biol. 358(4):1179 - 90]. A single kinetic model topology with common intermediate structures is observed that is independent of the concentration or type of monovalent cation that is present during folding. The differences in the time-progress curves at the different ionic conditions are accommodated by repartitioning of the folding flux among the available folding pathways. Introduction of a severely destabilizing mutation changed the structure of the intermediates but not the kinetic model topology, demonstrating that the native state topology dictates the structures of the folding intermediates. The effect of initial RNA conformation and electrostatic environment upon folding on flux partitioning was distinguished from folding experiments in which the monovalent cation and Mg2+ concentrations were concurrently adjusted. The resultant kinetic models show that whereas native state topology determines the intermediate structures, their lifetime and relative abundance are dependent on either or both the initial conformational ensemble or the folding reaction conditions.